(Art. 34 amendment)

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International Patent Application PCT/EP03/14542
Prof. Dr. Werner Seeger

In response to the Written Opinion of October 6, 2005

New claims 1-29 are filed herewith and it is requested to continue the International Preliminary Examination on the basis of this new set of claims.

1. Claim amendments

The new claims 1-29 corresponds to original claims 1-29 which have been amended as follows (see also the attached mark-up copy in which the changes made relative to the original set of claims are indicated).

In claim 11 the non mammalian plasminogen activators have been deleted.

In claim 12 and 13, respectively, reference has been now been made to the polypeptide sequences of SEQ ID NO: 19 and SEQ ID NO: 20 (claim 12), and to SEQ ID NO: 25 and SEQ ID NO: 26 (claim 13) in order to be in accordance with the sequence listing submitted on March 18, 2004.

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2. Patentability

In the Written Opinion, the following 4 references have been cited with regard to the patentability of the subject matter of original claims 1-29:

D1: Ruppert, C. et al. (2003) *Thromb. Haemost.* **89**, 53-64.
D2: Lindbladh, C. et al. (1993) *Trends Biochem. Sci.* **18**, 279-283.
D3: Peter, K. et al. (2003) *J. Cardiovasc. Pharmacol.* **42**, 237-244.
D4: Kerr, D.E: et al. (1999) *Bioconjug. Chem.* **10**, 1084-1089.

The novelty of the entire claimed subject matter has been explicitly acknowledged in the Written Opinion. However, the existence of an inventive step has been denied. According to the Examiner, the difference between reference D1 and the subject matter of claim 1 or claim 6 for example, is that in the present invention the mammalian surfactant protein is fused to the mammalian plasminogen activator instead of being cross-linked as in D1. The examiner considers this difference obvious in view of a combination of reference D1 with any of the reference D2 to D4.

We respectfully disagree with the examiner's opinion based on the following reasons.

The object of the present invention can be seen in providing a molecular tool/novel compound that is suitable for a fibrinolytic therapy against surfactant-containing fibrin and thus is suitable for treatment of acute inflammatory and chronic interstitial lung diseases. It is correct that in this regard D1 can be seen as closest prior art for assessing the inventive step of the invention claimed in the present application.

2.1. Reference D1

It has to be noted in this regard that according to D1 the conjugate in which urokinase is chemically cross-linked to the pulmonary surfactant protein B exhibits favourable property compared to an earlier construct consisting of a monoclonal antibody against surfactant protein B (SP-B) and urokinase.

As explained in D1 (see page 62, left column, line 6ff), coupling of urokinase and SP-B represents the more promising approach than antibody conjugate with SP-B since the purified hybrid molecule of D1 was shown to possess distinct advantages that could make it a preferable plasminogen activator for induction at the alveolar level. Firstly, according to D1, in addition to the

fibrinolytic function, the biophysical properties of SP-B were fully maintained. Second, due to the maintenance of the surface activity, easy spreading of the hybrid molecule of D1 at the alveolar surface can be anticipated. Thirdly, the hybrid molecule of D1 was shown to be about 2-3 times more effective than the earlier antibody conjugate in fibrinolysis of surface containing clots. And fourthly, the hybrid molecule of D1 turned out to be more resistant than the native urokinase molecule and more active since higher inhibitor concentrations of the urokinase inhibitor PAI-1 were necessary to gain the same agree of inhibition. D1 then concludes that the chemically cross-linked conjugate of D1 of urokinase with pulmonary surfactant protein B was proven not only to be superior to urokinase but also to the recently described urokinase anti-SP-B antibody conjugate.

Accordingly, D1 as such does not provide any motivation to modify the chemically cross-linked conjugate of D1 of urokinase with pulmonary surfactant protein B and to arrive at the fusion proteins of claim 1 and claim 6, respectively. Therefore, the fusion protein of independent claims 1 and 6 is already inventive over D1 alone.

2.2. Reference D2

Document D2 is concerned with the comparison of recombinant and synthetically formed conjugates of monoclonal antibodies with β-lactamase for anticancer prodrug activation. Thus, D2 only relates to the use of such a fusion protein in cancer therapy (see for example, abstract of D2). There is no reference at all in D2 to plasminogen activators or the treatment of acute inflammatory and chronic interstitial lung diseases. Thus, the skilled person would not have any motivation to combine the teachings of document D1 with that of reference D2. Accordingly, the fusion protein of claim 1 and claim 6 are inventive in view of D1 and D2.

2.3. Reference D3

Document D3 describes the construction and in vitro testing of a fusion protein consisting of a Fab fragment of the fibrin-specific monoclonal antibody 59D8 and hirudin. The aim of these experiments is to learn about the suitability of this fusion protein of hirudin for (potential systemic application) as a new anticoagulative therapeutic agent. In this conjunction, D3 compares the fusion protein comprising the 59D8 Fab antibody fragment and hirudin with a known single chain antibody hirudin fusion protein. The advantages of the fusion protein of D3 over this known fusion protein are mainly seen in choosing the Fab fragment as format of the antibody (binding site). Thus, also D3 does not relate at all to the field of plasminogen activators or the treatment of acute inflammatory and chronic interstitial lung diseases.

2.4. Reference D4

Reference D4 discusses the use of fusion proteins of polypeptides such as antibodies, antibody fragments or protein A for their use in enzyme immunoassays (see for example, Table 1, page 281 of D4). Also D4 is not concerned at all with plasminogen activators or the treatment of acute inflammatory and chronic interstitial lung diseases.

Thus, there is no motivation for the person skilled in the art to combine reference D1 with any of the references D2 to D4 when in search of a new molecular tool that is suitable for a fibrinolytic therapy against surfactant-containing fibrin. Accordingly, the fusion protein of claim 1 and claim 6, respectively, is inventive over the cited references already for this reason.

2.5. Properties of mammalian surfactant proteins/new experimental data

In this conjunction it should be noted that the person skilled in the art would not have any motivation to use either a mammalian surfactant protein precursor lacking its C-terminal propeptide or a mature mammalian surfactant protein in a fusion protein with a plasminogen activator due to following reasons.

With regard to the mature mammalian surfactant protein precursor and claim 1, it had to be assumed that the propeptide of surfactant protein B and C (SP-B and SP-C) prevents the mature surfactant protein from exhibiting its biophysical activity during the delivery to the alveolar cells, meaning that the propeptide provides a "shield" against the (at that time of protein transport highly detrimental) function and cell damaging properties of the mature surfactant protein (see, page 6, lines 1-4 of the present application). Therefore, the skilled person would not have been motivated to modify the chemically cross-linked conjugate of D1 - also not in view of any of D1 to D3 - and to use such a propeptide for the construction of a fusion protein with a plasminogen activator. Also for this reason, the fusion protein of claim 1 is inventive over the cited references D1 to D4.

With regard to the use of a mature surfactant protein and in particular mature SP-B in a fusion protein as defined in claim 6 or 10, we would like to note that - as the examiner also correctly states in the Written Opinion in point 9 under "certain observations" - recombinant production of mature SP-B alone has not been possible and is still not possible. Due to its hydrophobicity, expressed mature SP-B will immediately fuse with/disrupt lipid membranes which in turn results in lysis of the cell that is used for recombinant production (cf. page 5, lines 31to 35 of the present application). Thus, the person skilled in the art would have no motivation to generate a fusion

protein of mature SP-B with a plasminogen activator. Accordingly, the use of mature SP-B in such a fusion protein and the successful production of active fusion proteins of SP-B is by no means obvious but surprising and thus inventive in view of the prior art. As further support, we would also like to refer to the results of the experimental data which are submitted herewith as Exhibit A and Exhibit B.

2.5.1 Exhibit A: recombinant protein production in CHO cells

As can be seen from these Exhibit A, the inventors recombinantly produced in CHO cells four different fusion proteins encoding mature SP-B and low molecular weight urokinase (u-PA) using the mammalian expression vector pcDNA3.1. This four fusion proteins are

1. N-term SP-B/C-term u-PA: mature SP-B fused to the N-terminus of u-PA
2. N-term SP-B/C-term u-PA: mature SP-B fused to the N-terminus of u-PA + histidine tag
3. N-term u-PA/C-term SP-B: mature SP-B fused to the C-terminus of u-PA,
4. N-term u-PA/C-term SP-B: mature SP-B fused to the C-terminus of u-PA, + histidine tag.

Urokinase activity was found in supernatants and lysates of transfected CHO cells with highest activities being found for the N-term u-PA/C-term SP-B construct. Furthermore, the recombinant fusion protein (SPUC) was detected in the supernatant by western blotting and casein enzymography, showing that recombinant production of a fusion protein consisting of mature SP-B and u-PA is best, when the SP-B moiety is fused to the C-terminus of urokinase.

2.5.2 Exhibit B: Generation and investigation of transgenic mice

In addition and as shown in Exhibit B, the inventors generated transgenic mice that expressed the fusion protein in which mature SP-B is fused to the C-terminus of u-PA.

The successful generation of transgenic mice expressing the SP-B-urokinase fusion protein was documented by the detection of : a) transgene-specific SP-B-urokinase mRNA in the lung homogenate by RT-PCR, b) SP-B-urokinase-protein in the bronchoalveolar lavage fluid by western blotting using antibodies against SP-B and urokinase, c) increased proteolytic activity in the bronchoalveolar lavage fluid as assessed by a colorimetric assay and d) a lysis zone corresponding to the predicted size of the fusion protein as assessed by casein zymographie. Long-term induction of transgene expression revealed that double transgenic mice are healthy, fecund and indistinct from un-induced double transgenic mice and from single transgenic and wild type littermates.

In addition, it was demonstrated that the expression of a SP-B-urokinase fusion protein in the distal respiratory epithelium improves survival in acute lung injury induced by aerosol LPS administration. Similarly, survival was also improved after aerosol bleomycin challenge. The bleomycin model of lung fibrosis is based on an early ARDS-like acute pulmonary inflammation, followed by a subsequent fibrosis of the lung. As evident from the survival studies the majority of wild type and low/medium expressing mice died in the fibroproliferative phase. This provides evidence that the improved survival in high expressing transgenic mice after bleomycin challenge is the result of an attenuation of the fibrotic response in this model. In line with this suggestion all criteria, indicating the development of lung fibrosis, were markedly improved in the high expressing transgenic mice: lung compliance was increased, lung tissue hydroxyproline was virtually normalized and histological abnormalities in the lung were clearly reduced.

In summary, the transgenic mice expressing the SP-B-urokinase fusion protein of the invention are protected against acute lung injury and post-inflammatory fibrosis. Accordingly, the production of a recombinant surfactant protein B-urokinase fusion protein and its application in acute lung injury and pulmonary fibrosis experimental and clinical studies presents a promising therapeutic strategy in the future.

For the above reasons, the Examiner is kindly requested to reconsider his concerns and to acknowledge the existence of an inventive step for the fusion protein of claims 1 and 6 and thus for the entire claimed subject matter.

3. Clarity of the claims/Support by the description

Due to the deletion of the non mammalian activators from claim 11 and due to the amendments of the SEQ ID NO: in claims 12 and 13, the respective clarity objection in the section "Certain observations" are made moot.

Likewise, in view of the experimental results of Exhibits A and V, the objection in point 9 that claim 22 lacks support within the meaning of Art. 6 PCT and the description lacks disclosure within the meaning of Art. 5 PCT should also be overcome.

The examiner is thus kindly requested to issue a fully positive International Preliminary Examination Report. Should he see any need for clarification in this regard, he is kindly requested

to contact the representative by phone or to discuss the matter in a personal interview. Alternatively, issuance of a second Written Opinion pursuant to Rule 66 PCT is requested.

Dr. Wolfram Schiweck
Patentanwalt

Encls.
New claims 1-29 (clean and mark-up copy),
Exhibit A (experimental data),
Exhibit B (experimental data)

New Claims

1. A fusion protein comprising:

(a) a mammalian surfactant protein precursor lacking its C-terminal propeptide, and

(b) a mammalian plasminogen activator,

wherein the surfactant protein precursor is fused at its C-terminus to the N-terminus of the plasminogen activator.

2. The fusion protein of claim 1, wherein one of the protein components (a) or (b) is a human protein.

3. The fusion protein of claim 1 or 2, wherein both protein components (a) and (b) are human proteins.

4. The fusion protein of any of claims 1 to 3, wherein the surfactant protein precursor is selected from surfactant protein B (SP-B) or surfactant protein C (SP-C).

5. The fusion protein of any of claims 1 to 4, wherein the surfactant protein precursor is surfactant protein B (SP-B).

6. A fusion protein comprising:

(a) a mature mammalian surfactant protein, and

(b) a mammalian plasminogen activator,

wherein the mature surfactant protein is fused at its C-terminus or its N-terminus to the N-terminus or the C-terminus of the plasminogen activator, respectively.

7. The fusion protein of claim 6, wherein one of the protein components (a) or (b) is a human protein.

8. The fusion protein of claim 6 or 7, wherein both protein components (a) and (b) are human proteins.

9. The fusion protein of any of claims 6 to 8, wherein the mature surfactant protein is selected from the group consisting of surfactant protein B (SP-B), and surfactant protein C (SP-C).

10. The fusion protein of any of claims 6 to 9, wherein the mature surfactant protein is surfactant protein B (SP-B).

11. A fusion protein of any of claims 1 to 10, wherein the mammalian plasminogen activator is selected from the group consisting of high molecular weight two-chain urokinase-plasminogen activator (HMW-u-PA), low molecular weight two-chain u-PA (LMW-u-PA), low molecular weight u-PA B-chain, recombinant single-chain u-PA (r-scu-PA), tissue-plasminogen activator (t-PA), recombinant t-PA (rt-PA), its variants r-PA, n-PA, and TNK-t-PA, and catalytically active mutants of the plasminogen activator.

12. The fusion protein according to any of claims 1 to 5 comprising the surfactant protein B (SP-B) precursor N-terminally fused to the low molecular weight two-chain u-PA (LMW-u-PA), as shown in SEQ ID NO: 19 and SEQ ID NO: 20, respectively.

13. The fusion protein according to any of claims 6 to 10 comprising the mature surfactant protein B (SP-B) fused to the low molecular weight two-chain u-PA (LMW-u-PA), as shown in SEQ ID NO: 25 and SEQ ID NO: 26, respectively.

14. The fusion protein of any of claims 1 to 13, which carries a protein or peptide affinity tag at its N-terminus and/or at its C-terminus.

15. A nucleic acid molecule comprising a nucleotide sequence encoding a fusion protein of any of claims 1 to 14.

16. The nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 6 or SEQ ID NO: 7.

17. The nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 12 or SEQ ID NO: 13.

18. The nucleic acid molecule according to any of claims 15 to 17, wherein the nucleic acid molecule is operably linked to a regulatory sequence to allow expression of the nucleic acid molecule.

19. The nucleic acid molecule according to claim 18, wherein the regulatory sequence comprises a promoter sequence and a transcription termination sequence.

20. The nucleic acid molecule of any of claims 15 to 19 comprised in a vector.

21. A host cell containing a nucleic acid molecule of any of claims 15 to 20.

22. A method for production of a fusion protein of any of claims 1 to 14, comprising:

(a) introducing a nucleic acid molecule encoding the fusion protein into a suitable vector, and

(b) introducing the recombinant vector obtained in (a) into a suitable host cell or into a suitable cell extract.

23. A pharmaceutical composition comprising a fusion protein of any of claims 1 to 14.

24. Use of a fusion protein of any of claims 1 to 14 for the manufacture of a pharmaceutical composition.

25. The use of claim 24, wherein the pharmaceutical composition is for prevention and/or treatment of inflammatory and interstitial lung diseases.

26. The use of claim 24 or 25, wherein the pharmaceutical composition has fibrinolytic activity.

27. A method of prevention and/or treatment of inflammatory and interstitial lung diseases, comprising the step of administering a fusion protein of any of claims 1 to 14 to a mammal at a dose sufficient to prevent and/or treat the disease.

28. The method according to claim 27, wherein the fusion protein is administered to a mammal by an administration selected from the group consisting of parenteral administration, non-parenteral (enteral) administration, and topical administration.

29. The method according to claim 28, wherein parenteral administration is by aerosol administration or intratracheal instillation.